HOST FISH ASSESSMENT AND GRAVIDITY FOR THE MUSSEL ELLIPTOIDEUS SLOATIANUS

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Host Fish Assessment and Gravidity for the Mussel *Elliptoideus sloatianus*

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I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

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Summary

Elliptoideus sloatianus (purple bankclimber) is a freshwater mussel that is endemic to the Apalachicola River Basin, which includes the Chattahoochee, Flint, Chipola, and Apalachicola Rivers. Populations of *E. sloatianus* also exist in the Ochlockonee River, which discharges independently from the ACF basin into the Gulf of Mexico. In 1998, *E. sloatianus* was listed as federally threatened due to the loss of suitable habitat and the potential blockage of host fish passage into areas where the mussel lives. For this study, we located three populations of *E. sloatianus* in the Flint River in southwest Georgia. Gravid mussels were collected in late winter through early spring when the river was above normal flow. The early collection time allowed for fertilization in the river and glochidial development in the lab. To determine the larval host fish of *E. sloatianus*, trials were conducted using 16 species of fish, 7 of which successfully transformed *E. sloatianus* glochidia to the juvenile stage. *Percina nigrofasciata* (blackbanded darter) transformed significantly more juveniles than the other 6 species, suggesting it is potentially a primary host. In addition, the period of gravidity for *E. sloatianus* was determined to be late-March to mid-June by weekly observations at 3 study sites on the main-stem Flint River located in southwest Georgia. Cages were employed to hold some mussels at the sites where they occurred to ease monitoring. Surveys showed that *E sloatianus* individuals were found in areas of the main-stem river that provided a stable substrate and moderate to high stream flows.

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Chapter 1

Abstract

Assessments of the factors that affect the sustainability of freshwater mussel populations from the family Unionidae have become important due to the large number of species that are endangered, threatened or in decline. Because larval freshwater mussels are dependent on fish, a key component of freshwater mussel conservation is host fish species identification. The primary objective of this study was to identify potential host fish for the federally threatened mussel *Elliptoideus sloatianus* (purple bankclimber). The mussels used in this study were taken from populations located in the Flint River within the Apalachicola-Chattahoochee-Flint River Basin (ACF). The 16 potential host fish in this study are sympatric with *E. sloatianus* in the ACF and included species examined in previous studies. The effectiveness of the fish as hosts was evaluated using laboratory infections. Seven fish species successfully transformed glochidia into juveniles, suggesting that they were potential hosts. Juveniles were transformed on fish species not seen in previous studies and, conversely, some fish species that were apparent hosts in previous studies did not serve as hosts in the present study. Host fish studies are necessary for the successful propagation and intensive culturing of freshwater mussels, providing a means for the augmentation of threatened populations and re-introduction of mussels into sites where they have been extirpated.

Introduction

The rivers of the southeastern United States contain one of the most diverse assemblages of freshwater mussels in the world, with some locations historically supporting more than 70 species (Nedeau et al. 2009). Mussels are also highly imperiled, as 71% of the species are either federally listed or candidates for listing (Williams 1993, Haag and Warren 2003). Their decline, which has been documented in several studies (e.g. Jorgenson and Sharp 1971, Szymanski 1998, Watters 1999), has been attributed to a variety of causes, including increased siltation, toxic chemical waste, channelization of rivers, and the damming of rivers and streams (Neves et al. 1997).

Mussels play an important role in freshwater ecosystems, but basic life history information is lacking for about 90% of the species found in the Southeast (Neves et al. 1997). Male mussels release sperm into the water column that are taken in by females through their incurrent apertures (Watters 1995). Within the gills of a female mussel, fertilized eggs develop into larvae, called glochidia. Unionid mussels are divided into two groups based on when the females release the glochidia (Watters 1995). Bradytictic (long-term breeders) hold their larvae throughout the winter until the following spring or summer (Watters 1995). Tachytictic (short-term breeders) mussels release their larvae later the same year, usually by July or August (Watters 1995). Upon release, glochidia must parasitize specific species of fish by attaching to the gills, fins, or body. Once they are attached, the glochidia will become encysted and undergo transformation into juveniles (Williams et al. 2008). After a period of days to weeks, depending on the mussel species, they will excyst from the gills or fins (Williams et al. 2008) and drop off onto the substrate thus completing the transformation phase of their lifecycle.

Data on the relationship between listed mussels and their larval host fish is limited but critical in conservation efforts, since reintroductions and augmentation of mussel populations are only successful if a mussel species' host fish is present in the same areas as the mussel (Farzaad 1991). Some mussels are highly host-specific and are referred to as specialists, while others are less host-specific and considered generalists (Zales and Neves 1982, Yeager and Neves 1986). In some cases, different populations of the same mussel species may not use the same species of host fish (Rogers 1999). The reasons for differences in the susceptibility of fish to parasitism by mussel species are not well-understood, but the most likely cause is the immune responses of the host (Zales and Neves 1982, O'Connell and Neves 1999).

There are advantages and disadvantages to using laboratory studies or field studies to assess mussel-host fish relationships. Field observations of natural infections may not necessarily show whether successful transformations have occurred, because the glochidia may become excysted from the fish before transformation to the juvenile stage and be missed during field observations (Barnhart et al. 2008). Additionally, identifying the glochidia attached to the gills is difficult, and if attachment is recent, the fish's immune system may not yet have had time for the eventual rejection of the glochidia (Barnhart et al. 2008). Therefore, lab studies are needed to confirm such transformations. On the other hand, lab studies may indicate host relationships that seldom or never occur in nature, especially when non-native fish species are used.

Elliptoideus sloatianus, or the purple bankclimber, is a mussel endemic to the Apalachicola-Chattahoochee-Flint (ACF) Basin of Alabama, Georgia, and Florida, and the Ochlockonee River in Florida and Georgia (Figure 1; Lea 1840, Clench and Turner

1956, Burch 1975). It is the 2nd largest mussel in the ACF basin and appears to favor sand, fine gravel, or muddy sand substrates, in the moderate current of large rivers (Lea 1840, Clench and Turner 1956, Heard 1975). *E. sloatianus* was classified as rare by Clench and Turner (1956), endangered by Athearn (1970) and Stansberry (1971), and threatened by Williams et al. (1993) and Williams and Butler (1994). It was proposed for federal threatened status in 1994 and listed in 1998 (USFWS 1998).

Several studies have examined potential host fish for *E. sloatianus*. In the laboratory, O'Brien (1997) infected 14 fish species and successfully transformed glochidia into juveniles on 3 of the fish species, the mosquito fish (*Gambusia affinis)*, guppy (*Poecilia reticulata)* and blackbanded darter (*Percina nigrofasciata)*. However, due to low transformation rates, O'Brien did not consider these species as likely primary hosts (O'Brien 1997, Brim-Box and Williams 2000). Johnson (pers.comm.), in a laboratory trial, had modest success producing a single juvenile on a greater jumprock (*Moxostoma lachneri*) but stated that the overall viability of the glochidia used in the procedure appeared low. Therefore, *M. lachneri* is only classified as a secondary host for *E. sloatianus* (Williams et al. 2008).

Therefore, the objective of this study was to identify likely host fish species for *E. sloatianus* using laboratory techniques, leading to the production of juvenile mussels. Native fish sympatric with *E. sloatianus* in the Flint River in Georgia were used as potential hosts. These species are generally active at or near the stream substrate, making them the species most likely to encounter conglutinates of *E. sloatianus* which, upon release, settle to the substrate in the absence of stream flow. The mussels were taken from the Flint River after fertilization to increase the likelihood of viable glochidia. By

determining the likely host fish of *E. sloatianus*, this project provides valuable information to assist in population augmentation of a threatened mussel species.

Methods

Study site

The study was conducted at the Warm Springs National Fish Hatchery in Warm Springs, Georgia. Two ponds, each with a surface area of 1,012 m^2, supplied soft water to the mussel facility at a rate of up to 190 liters/minute. Spring water from Cold Springs Creek was supplied to the ponds and re-circulated while being treated. Infection trials were conducted in the hatchery mussel building where water temperatures were manipulated to mimic seasonal changes seen at three field sites along the Flint River. Mussels were collected from the Flint River at Lake Worth Dam, Dougherty County, Albany Georgia (N 31. 60087°, W 84.13905°).

Host fishes

The collection of host fish began in the summer 2009 and continued through spring 2010. The 16 species chosen are commonly found in areas inhabited by *E. sloatianus* (B. Birkhead, pers. comm.). To preclude any immunity issues (Preister 2008), fishes were collected from tributaries and areas within the main-stem of the Flint River without any *E. sloatianus.* Additional fish were collected from a tributary of the Chattahoochee River that was devoid of any mussels. Five individuals from each of the following 13 fish species were used in the experiment: *Moxostoma sp.cf. poecilurum* (grayfin redhorse), *Minytrema melanops* (spotted sucker), *Lepomis auritus* (redbreast sunfish), *Lepomis*

macrochirus (bluegill), *Cyprinella venusta* (blacktail shiner), *Notropis longirostris* (longnose shiner), *Notropis buccatus* (silverjaw minnow), *Notropis texanus* (weed shiner) *Ictalurus punctatus* (channel catfish), *Noturus leptacanthus* (speckled madtom), *Percina nigrofasciata*, (blackbanded darter), *Micropterus cataractae* (shoal bass), and *Micropterus salmoides* (largemouth bass). A single individual of three additional fish species were also used: *Lepomis gulosus* (warmouth), *Moxostoma lachneri* (greater jumprock) and *Micropterus punctulatus* (spotted bass).

Once collected, the fish were held in quarantine for 30 days (USFWS 2007). The fish underwent a constant salt treatment at 0.05% NaMg per 3.8 liters of water to remove any external parasites. *P. nigrofasciata* were treated at a lower salt concentration, 0.025% NaMg per 3.8 liters of water, to reduce mortality during the quarantine process (Marking et al. 1994). Following quarantine, fish were transferred to the fish-host system and held until the start of the infection process (USFWS 2007).

Mussel collection

Starting in May 2009, three field sites in the ACF basin in Georgia were visited each month to check for gravidity of local *E. sloatianus*: Lake Worth Dam, Dougherty County (N 31.60087°, W 84.13905°), Philema Shoals, Lee County (N 31.72408°, W 84.01906°), and Montezuma Bluffs, Macon County (N 32.33664°, W 84.02978°). In early spring 2010, once gravid mussels were found, they were transported to Warm Spring National Fish Hatchery using established protocols (USFWS 2007). These mussels were the source of glochidia used for the host fish infections.

Viability of *E. sloatianus* conglutinates

When *E. sloatianus* mussels released conglutinates that contained fertilized eggs, these were removed and placed in a beaker where they broke apart easily, suggesting they were mature. From each release, one conglutinate was placed in a Petri dish and examined under a dissecting scope for quality and maturity of glochidia present. Fully developed conglutinates showed approximately 95% glochidia and 5% eggs. Glochidia also opened and closed repeatedly, termed a "glochidial snapping action". A small subsample of the glochidia was exposed to a saline solution. If at least 50% of the individuals snapped shut in response, the glochidia were considered viable (Gordon 2001). The number of glochidia per conglutinate was estimated by suspending the glochidia in 1L of water and agitating them with a pipette. While stirring, ten 1mL subsamples were removed with a volumetric pipette, and glochidia from each subsample were counted under magnification on a plankton wheel, to estimate total numbers of glochidia per volume of sample. The average number in the samples was multiplied by 1000 to estimate the number of glochidia in the 1L suspension volume.

Fish infection procedures

Inoculation of fish adhered to the following protocol: individuals or groups of fish were placed in a container containing glochidia. An airstone was placed in the water to keep the glochidia suspended in the water column. The density of glochidia in each individual bath was controlled by adding water until the desired concentration of 4000 glochidia/liter was achieved (Fobian 2007). The fish were suspended in the glochidia bath for 15 minutes. Following inoculation, the larger fish were manually examined for

glochidial attachment whenever possible by visually inspecting the gills to confirm successful infection. The remaining water in each infection container was poured through a 100 micron brass sieve, and the collected (i.e. unattached) glochidia were quantified.

Once infected, fish were put into individual aquaria of different volumes (1.5L, 3.0L, or 37.85L) depending on the size of the fish. The aquaria were siphoned daily for the first 5 days post-inoculation to check for sloughed off glochidia and juvenile mussels (O'Brien and Williams 2002). Afterwards, aquaria were siphoned every 3rd day. When juveniles were found, they were counted and transferred into the juvenile holding system (Dodd et al. 2005; Barnhart 2006). Aquaria were siphoned until juveniles were no longer found for two consecutive siphoning events.

Juvenile mussels from each fish host species were combined and fed a 500 ml mixture of Shellfish Diet (Aquatic Ecosystems) and marine algae, *Nanochloropsis sp.* Juveniles were fed every 6 hours using a drip system with a delivery rate of 25-50 ml/min (Fobian, pers. comm.).

Results

Of the 16 fish species used in this study, 7 successfully transformed glochidia into juveniles (Table 1): *L. auritus, L. macrochirus, M. cataractae, M. salmoides, M. punctulatus*, *C. venusta*, *and P. nigrofasciata. P. nigrofasciata* transformed significantly more glochidia than the other species (1-way ANOVA, $F_{2,28}=6.34$, P=0.0003; Figure 2) which, in turn, did not differ from one another. The majority of the transformations occurred at 20-45 days post-infection with temperatures ranging from 15-22°C. Fish species that did not transform juveniles shed their glochidia within 7 days post-infection,

with *I. punctatus* shedding all glochidia within 3 days.

Elliptoideus sloatianus are tachytictic breeders, whose eggs are fertilized in late winter to early spring and are released as conglutinates in mid-spring and continue through summer. *E.sloatianus'* conglutinates were bright white and mimic the fry of a prey fish. On average, 214 conglutinates were released across the 10 mussels used in the host trials. The conglutinates contained an average of 17,500 glochidia. The conglutinates were released over an average period of 31 days.

Discussion

P. nigrofasciata were significantly better hosts than the other species in transforming glochidia--up to 6 times better than the other species that had successful transformations. *P. nigrofasciata* was also the only species of fish to transform glochidia in both past and present studies, suggesting its potential importance as a host for the persistence of *E. sloatianus* populations. O'Brien's (1997) study infected 6 *P. nigrofasciata*, though only one individual fish yielded both of the successful transformations. However, the present study produced an average of 22 juveniles on 5 fish, with a range of 0 to 35 transformations per fish. *P. nigrofasciata* has not previously been classified as a potential primary host, based on the criterion that one fish must produce at least 3 transformations (O'Brien 1997). Host studies for other mussel species suggest that this number is consistent with host designation based on the small size of darters (Zales and Neves 1982, Haag and Warren 2003). In the present study, *P. nigrofasciata* greatly exceeds this cut-off, suggesting that it is a potential primary host for *E. sloatianus*. However, given its small size, *P. nigrofasciata* may fit somewhere between a primary host and a marginal

host (Haag and Warren 2003).

Interestingly, juveniles transformed on fish species in this study that did not transform in previous studies (O'Brien 1997). Conversely, *Moxostoma lachneri,* a fish host on which juveniles transformed in previous studies (P. Johnson, pers. comm.), did not transform on them in the present study. It is unclear why some fish species were successful hosts for *E. sloatianus* but not others. In this study, 7 out of 16 host species transformed glochidia, and of these 7, 4 belong to the family Centarchidae. Shared characteristics among the 7 species of host fish for *E. sloatianus* are that they inhabit areas with swift riffles to pools (Mettee et al. 1996), likely bringing them into contact with *E. sloatianus*. The smaller fish species all share diet similarities, feeding on small to large aquatic insect larvae and terrestrial insects. Large fishes from the family Centarchidae tend to feed on insect larvae as juveniles and small fishes and crayfish as adults (Mettee et al. 1996). With these diets, the host fish in this study may be more likely to be attracted to conglutinates, seeing them as likely prey. These similarities suggest that all 7 host species share characteristics that inherently make them suitable as hosts for *E. sloatianus* but do not explain why the other 9 fish species used in the study were not successful as hosts.

New techniques and methods in juvenile mussel propagation have led to attempts at restoring declining or extirpated populations by releasing hatchery-cultured juvenile mussels into areas affected by low population sizes (Eckert and Pinder 2010). One goal of the present study was to acquire data related to the US Fish and Wildlife 2003 Recovery Plan for Mussels of the ACF Basin. This study confirmed that juveniles of *E. sloatianus* can be transformed in the laboratory at levels that are required to implement

augmentation or reintroduction plans (U.S. Fish and Wildlife Service, 2003). *P. nigrofasciata* also appears to be an effective host for these efforts in the lab. However, in nature, *P. nigrofasciata* co-exists with *E. sloatianus*, yet recruitment remains low. This suggests that a better host fish species may yet be found. One explanation is the main primary host may be a migratory fish species whose access is blocked by dams and habitat alteration and no longer has passage to upstream areas where these mussels are located. The presence of an inferior secondary host may explain, however, the collection of an occasional *E. sloatianus* juvenile.

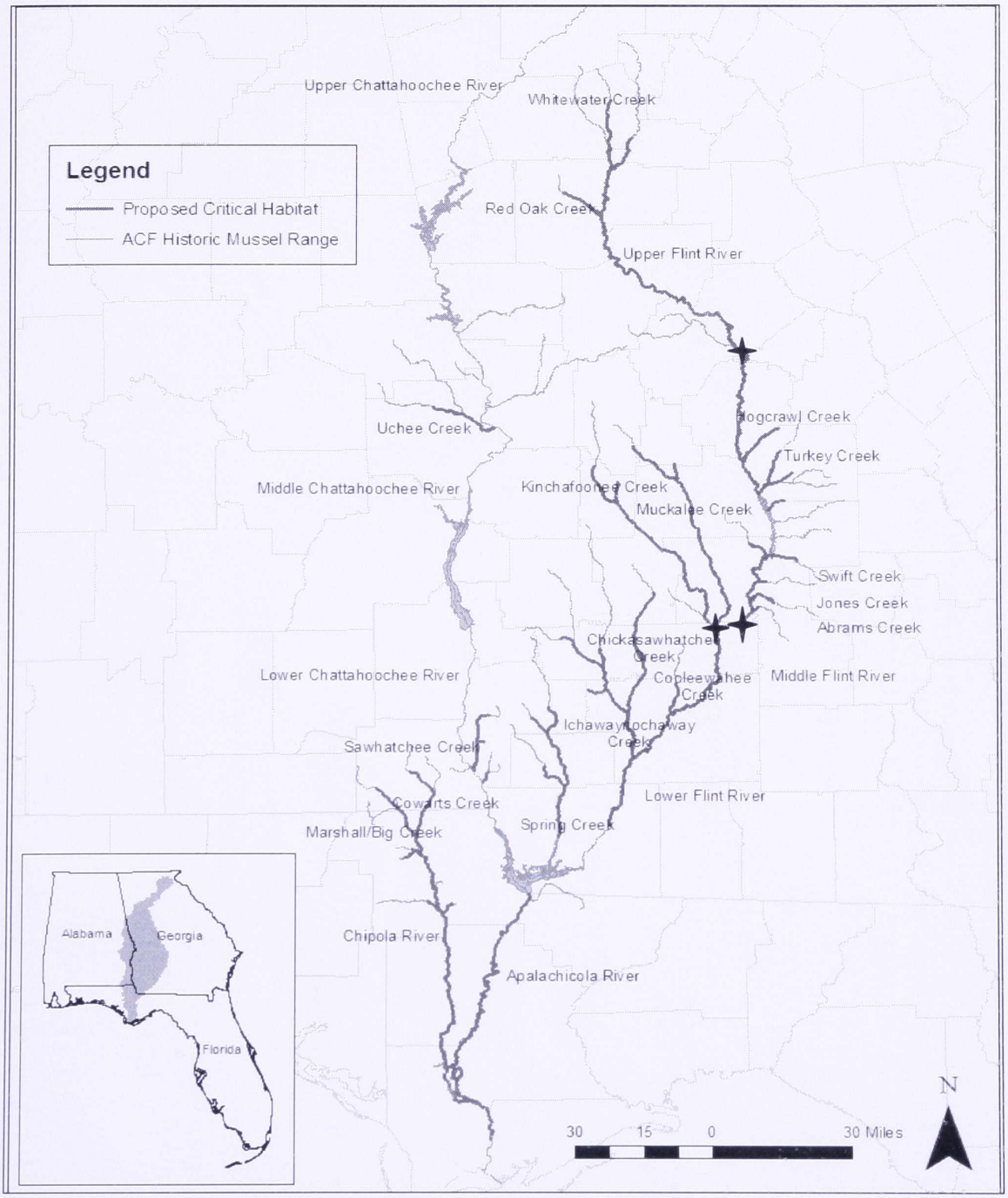

Figure 1 Map of the ACF drainages showing critical habitat and conservation areas represented by the thicker lines, proposed in 2003 by The US Fish and Wildlife Service in response to the 1998 federal listing of 7 mussel species within the ACF basin. The three 4-point stars represent the three gravidity study sites and the mussel collection site. (Map courtesy of U.S. Fish and Wildlife Service 2003 Recovery Plan for the Listed Mussels in the ACF)

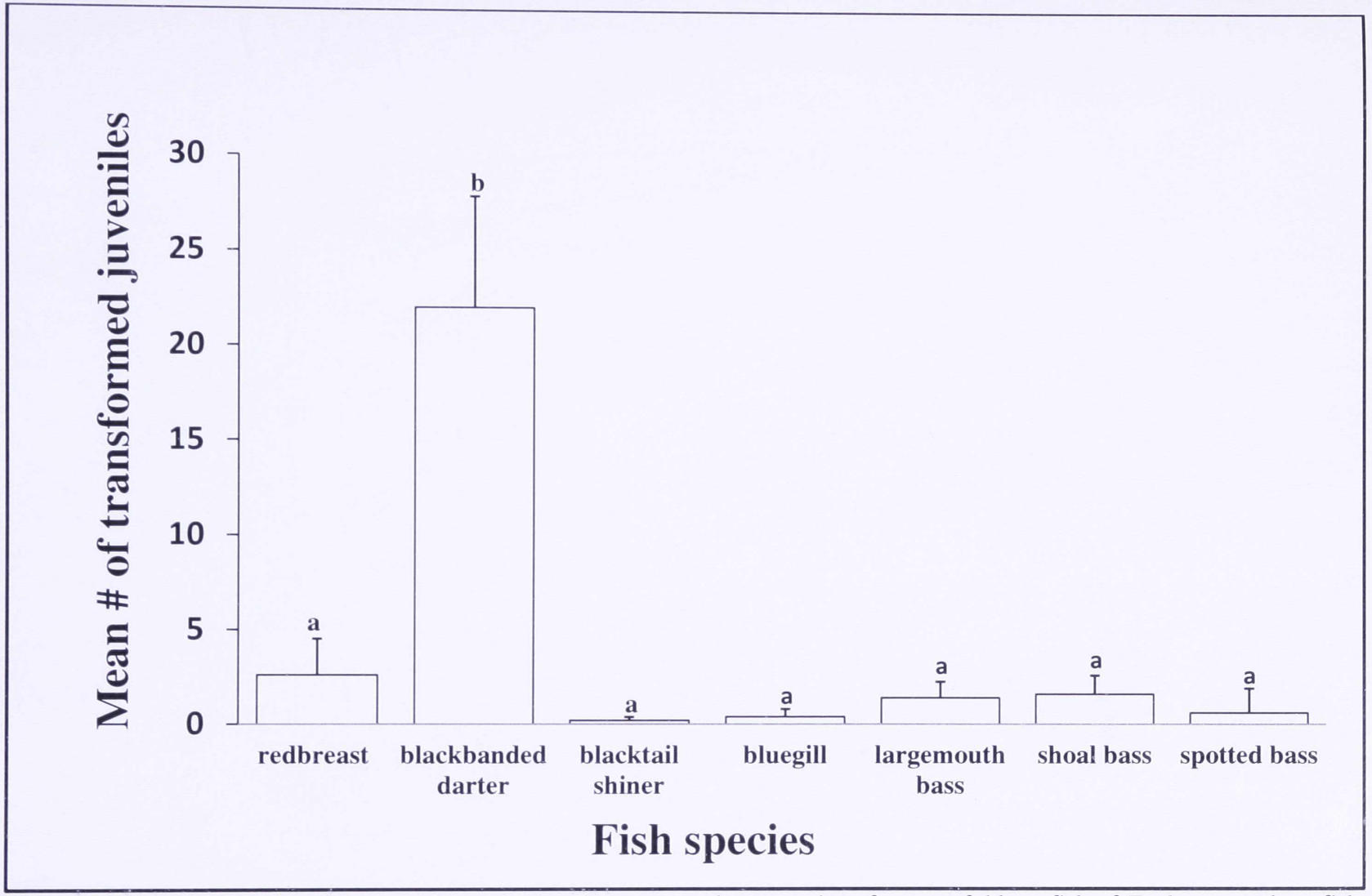

Figure 2 The average (+/- 1 S.E.) transformed juveniles recovered per species of successful host fish of *E. sloatianus* host fish trials at Warm Springs National Fish Hatchery from July 2009-July 2010. Bars sharing the same letter are not significantly different.

Table 1 The number of fish species infected with glochidia, total transformed juveniles per fish species, total glochidia recovered for fish species that transformed juveniles, mean and standard deviation (+/- 1 S.D.) per fish for the seven species of fishes that transformed juvenile mussels during host fish trials for the mussel *Elliptoideus sloatianus*.

Fish Species Infected	# of Fish Used	Total Transformed Juveniles	Total Glochidia Recovered	% Transformed	Mean Std. Dev. (+/-1 S.D.)
CASTOSTOMIDAE					
Moxostoma lachneri	1	0	0	0	0
Moxostoma sp.cf. poecilurum	5	0	0	0	0
Minytrema melanops	5	0	0	0	0
CENTARCHIDAE					
Lepomis auritus	5	13	264	4.92	2.6 (+/- 4.3)
Lepomis gulosus	1	0	0	0	0
Lepomis macrochirus	5	2	232	0.86	0.4 (+/- 0.9)
Micropterus cataractae	5	8	506	1.58	1.2 (+/- 2.2)
Micropterus salmoides	5	7	502	1.39	1.4 (+/- 1.9)
Micropterus punctulatus	1	3	42	7.14	0.6 (+/- 1.3)
CYPRINIDAE					
Cyprinella venusta	5	1	36	2.77	0.2 (+/ - 0.4)
Notropis longirostris	5	0	0	0	0
Notropis buccatus	5	0	0	0	0
Notropis texanus	5	0	0	0	0
ICTALURIDAE					
Ictalurus punctatus	5	0	0	0	0
Noturus leptacanthus	5	0	0	0	0
PERCIDAE					
Percina nigrofasciata	5	110	223	49.3	22 (+/- 13.1)

Appendix

Table 2 Average monthly water quality parameters for the three study sites on the Flint River from July 2009 thru June 2010.

Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Year	2009						2010					
Lake Worth Dam												
Temp °C	29.2	28.8	21.6	16	10.7	9.8	9	9.2	15.5	22	24	31.5
pH	7	7.5	7	6.5	7	6.5	6.8	7	6.8	6.5	7	6.8
NH^3 (mg/l)	0.5	0.2	0	0	0	0	0	0	0	0.2	0.2	0
Alkalinity(ppm)	104	64	56	60	62	64	68	80	66	54	48	56
Hardness (ppm)	68	62	54	62	58	60	60	74	60	48	44	54
Nitrites (mg/l)	0.05	0	0	0	0	0	0	0	0	0	0.05	0.05
Montezuma Bluff												
Temp °C	26.2	28.7	21	15.7	11	9.5	9.2	9.6	13	20.5	23.5	29.5
pH	7	6.8	6.5	7	6.5	7	6.8	7	7	7	7	7
NH^3 (mg/l)	0.5	0.2	0	0	0	0	0	0	0	0	0.2	0.2
Alkalinity(ppm)	20	18	20	24	26	28	22	24	22	24	20	10
Hardness (ppm)	14	20	20	20	22	24	20	20	20	22	18	15
Nitrites (mg/l)	0.05	0	0	0	0	0	0	0	0	0.05	0.05	0
Philema Shoals												
Temp °C	26	27.7	20.8	15.1	10.2	9.7	9.5	9	12	21.5	23.8	29.5
pH	7.5	7	7	7	7	6.5	6.5	7	6.5	7	6.5	7
NH^3 (mg/l)	0.2	0.5	0	0	0.2	0	0	0	0	0.2	0.2	0.2
Alkalinity(ppm)	48	22	40	46	46	40	44	24	22	20	22	10
Hardness (ppm)	40	16	40	44	38	44	38	28	22	22	20	10
Nitrites (mg/l)	0	0.05	0	0.05	0	0	0	0	0	0	0.05	0

Table 3 The locations and total fishes collected from each site used in *E. sloatianus* host fish trials.

Fish Species	Fish Collecting Locations								
	Hannahatchee Creek, Stewart County	Chickasawhatchee Creek, Calhoun County	Mountain Creek, Harris County	Lindsey Creek, CSU campus	Hatchery Pond # 11	Hatchery Pond # 12	Kendall Creek Muscogee County	Flint River, Highway 18 Bridge, Meriwether County	Flint River Highway 36 Bridge, Meriwether County
CASTOSTOMIDAE									
Moxostoma lachneri								1	
Moxostoma sp.cf. poecilurum								5	16
Minytrema melanops								6	20
CENTARCHIDAE									
Lepomis auritus	12		17					5	12
Lepomis gulosus	1								
Lepomis macrochirus								8	
Micropterus cataractae								15	
Micropterus salmoides						22			
Micropterus punctulatus									1
CYPRINIDAE									
Cyprinella venusta	17								
Notropis longirostris	112						9		
Notropis buccatus							20		
Notropis texanus				34					
ICTALURIDAE									
Ictalurus punctatus					20				
Noturus leptacanthus	19								
PERCIDAE									
Percina nigrofasciata	32	17	5				12		

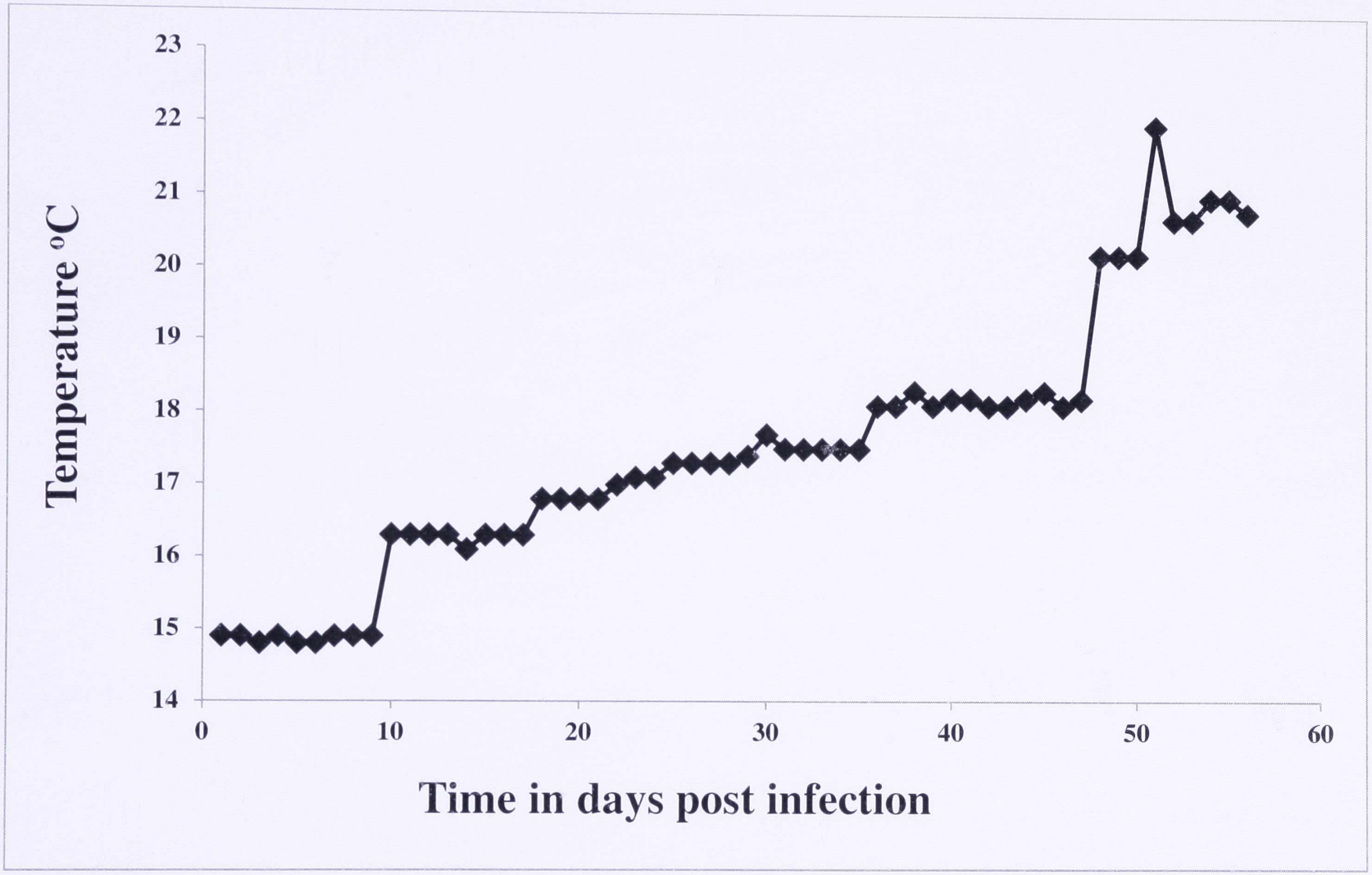

Figure 3 Daily temperatures in of the host fish system during the 60 day duration of the infection process in *Elliptoideus sloatianus* host fish trials. The increase in temperature over time is deliberate in an effort to mimic temperatures in the Flint River.

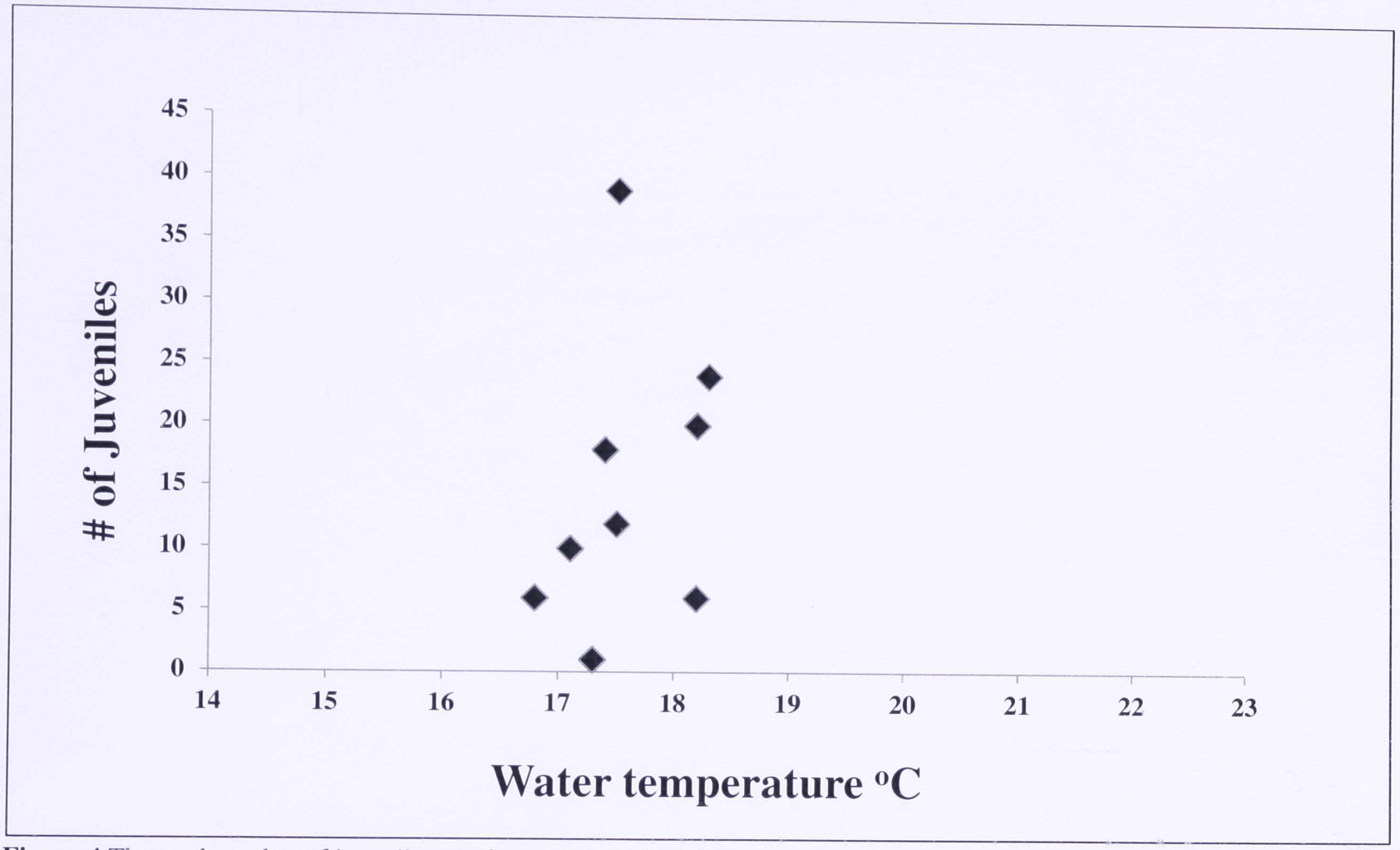

Figure 4 The total number of juvenile transformations in the laboratory across the 60 day range of temperatures in the mussel tank for *E. sloatianus* host fish trials at Warm Springs National Fish Hatchery from July 2009-July 2010.

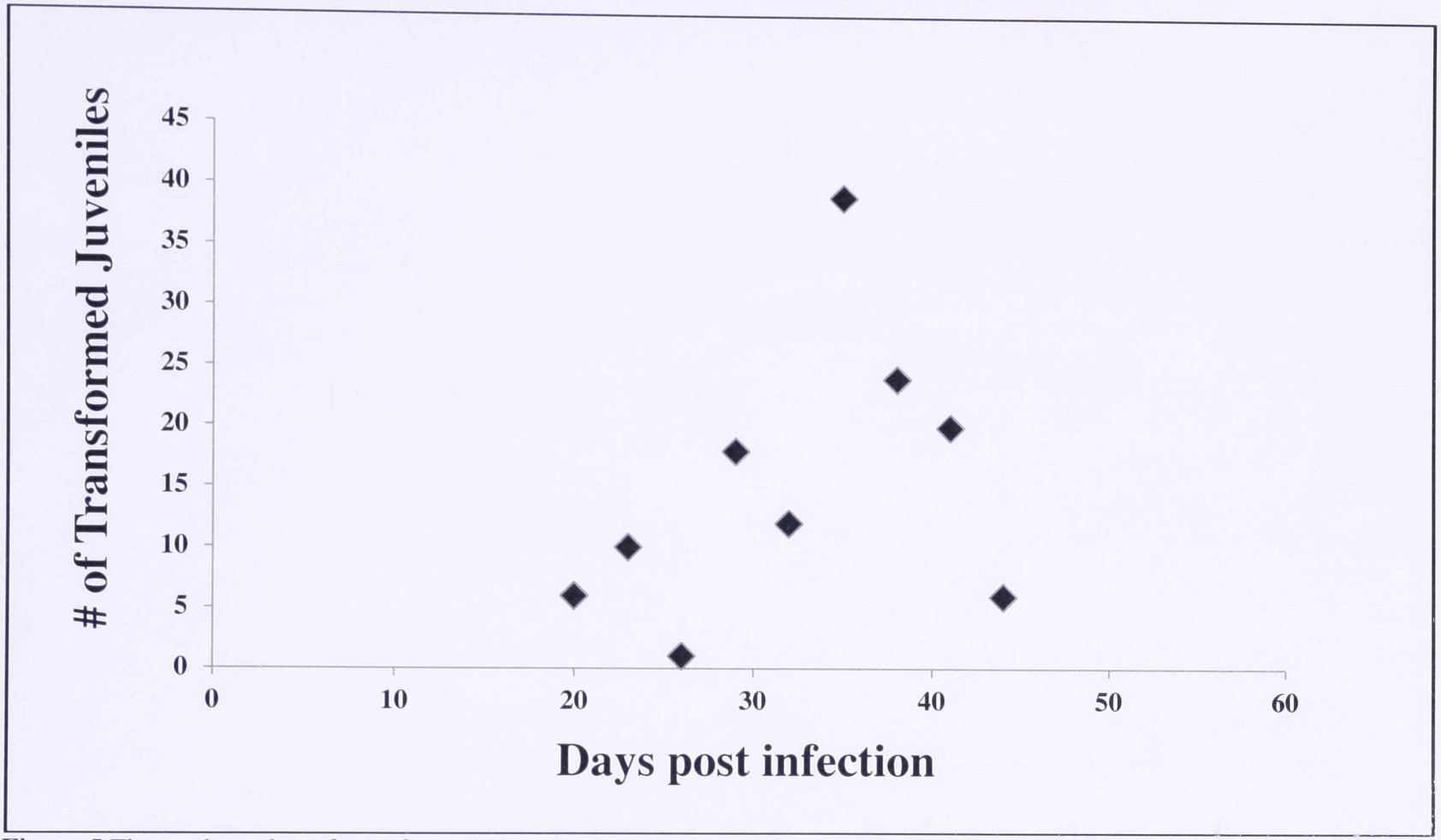

Figure 5 The total number of transformed juveniles per day for 60 days post infection across the seven species of fish that successfully transformed glochidia in *E. sloatianus* host fish trials at Warm Springs National Fish Hatchery from July 2009-July 2010.

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Gravidity for the Mussel

Elliptoideus sloatianus

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Chapter 2

Abstract

Understanding the interactions between mussels and their host fish is increasingly important as mussels experience reductions in population densities, recruitment and distribution. These reductions stem from several factors such as loss of habitat and decreases in water quality and quantity. For successful population reintroductions and augmentations, information is needed on species' life history traits and habitat preferences. Thus, the objectives for this study were to determine preferred habitats, document geographic distribution, and quantify the period of gravidity of the freshwater mussel *Elliptoideus sloatianus*, a federally threatened species. The populations of *E. sloatianus* examined in this study were located in the Flint River within the Apalachicola-Chattahoochee-Flint River Basins (ACF). Weekly for one year, mussels were inspected for gravidity. During the winter months when stream flows were elevated, mussels were placed in cages for easier access. Data on habitat characteristics and water quality parameters were collected during the weekly visits. Habitat preferences were determined to be moderate to high flowing water with stable substrate. *E sloatianus* was geographically widespread throughout the middle and lower Flint River. The period of gravidity for *E. sloatianus* was found to be from late-March through mid-June when water temperatures ranged from 15°C to 25°C. *E. sloatianus* on average, released 214 conglutinates over an average period of 70 days across the ten mussels used. Conglutinates were on average 2 cm long and 0.5 cm wide (n=10) and were two to three layers thick. Each of the 2 to 3 layer thick conglutinates averaged 17,500 glochidia, totaling on average 3.75 million glochidia per mussel.

Introduction

The diversity of freshwater mussels in the United States is the highest in the world, with 297 species and subspecies of Unionid mussels (Turgeon et al. 1988). Unfortunately, this assemblage is highly imperiled, with 71% of mussel species being either federally listed or candidates for listing (Williams 1993; Haag and Warren 2003). This decline of freshwater mussel populations has been documented in several studies (Jorgenson and Sharp 1971, Szymanski 1998) and has been attributed to a variety of causes, including increased siltation, releases of toxic chemicals, channelization of rivers, and dam construction (Neves et al. 1997).

One mussel that has been especially affected by these changes is *Elliptoideus sloatianus* (or purple bankclimber), an endemic to the Apalachicola-Chattahoochee-Flint (ACF) Basin of Alabama, Georgia, and Florida, and the Ochlockonee River in Florida and Georgia (Lea 1840, Clench and Turner 1956, and Burch 1975). Historical information indicates that *E. sloatianus* occurred in all three major ACF rivers. There is only one recent record from the Chattahoochee River drainage (Williams et al. 2008), but the species was present in three collections from Indian middens below the Fall Line in the Chattahoochee River drainage basin (Brim-Box and Williams 2000). Brim-Box and Williams (2000) also reported three historical records of *E. sloatianus* from Flint River tributaries. Heard (1975) noted that this species was common in the Apalachicola River in the 1960s, but by the mid-1970s the populations, especially below the Jim Woodruff Lock and Dam, had become drastically reduced. Brim-Box and Williams (2000), however, reported that *E. sloatianus* were locally abundant in the main channels of the Flint and Apalachicola rivers.

Because of its historical decline, *E. sloatianus* was classified as rare by Clench and Turner (1956), endangered by Athearn (1970) and Stansberry (1971), and threatened by Williams et al. (1993) and Williams and Butler (1994). It was proposed for federal threatened status in 1994 and listed in 1998 (USFWS 1998).

Despite the fact that it is critical for species conservation efforts, little is known about the life history and the reproductive biology of the majority of freshwater mussel species, including *E. sloatianus* (O'Brien and Williams 2002). Freshwater mussels have a complex life cycle with larvae that parasitize host fish (Layzer et al. 2003, Williams et al. 2008). Male mussels release sperm into the water column where they are taken up by an incurrent aperture in the females. Fertilized eggs develop into larvae known as glochidia that are released into the water column by one of a number of methods that serve to attract host fish (Williams et al. 2008). These methods include producing glochidia in bundles that resemble food items (conglutinates), producing superconglutinates of glochidia that are attached to the mussel by a long mucus string to mimic prey fish, discharging glochidia in a mucus web, holding glochidia in the gills with modified mantle flaps in the females to mimic small fish, and broadcasting individual glochidia into the water column (Williams et al. 2008). After the glochidia have attached to the host fish, they transform into juveniles, ex-cyst from the gills or fins, and drop off onto the substrate (Williams et al. 2008).

Given its threatened status and a dearth of life history information, the objectives for this study were to determine the periods of gravidity for *E. sloatianus*, along with their overall fecundity and the quantity of glochidia produced. The study also measured the duration of the viability of *E. sloatianus* conglutinates post-release.

Methods

Study sites and water characteristics

The study was conducted in the middle reaches of the Flint River and at Warm Springs National Fish Hatchery in Warm Springs, Georgia. Based on recent and historical mussel population survey data, three study sites for *E. sloatianus* were selected along the Flint River in Georgia (Figure 1): Lake Worth Dam, Dougherty County (N 31.60087°, W 84.13905°), Philema Shoals, Lee County (N 31.72408°, W 84.01906°), Montezuma Bluffs, Macon County (N 32.33664°, W 84.02978°). At all three sites, observations were made on water flow and the substrate where the mussels were found. Water quality conditions in the lab were maintained and manipulated to mimic natural parameters of the three field sites that were observed in the Flint River. During weekly visits, the following water quality parameters were measured: temperature, pH, NH3, alkalinity, hardness, and nitrites (Table 3).

Gravidity inspections

From July 2009 to July 2010, mussel populations were inspected for gravidity on a weekly basis. During these assessments, 3 to 10 mussels from each site were obtained and inspected for reproductive activity in the form of swollen gills and/or the presence of glochidia. In early spring 2010, once gravid mussels were found, 10 were removed from the cage at the Lake Worth Dam site and were transported to Warm Spring National Fish Hatchery using established protocols (USFWS 2007).

Water temperatures in the *E. sloatianus* tank mirrored river temperatures that were measured during the weekly visits. Each time a mussel released conglutinates, one was

placed in Petri dish and examined under a dissecting scope. Glochidia opened and closed repeatedly, with a quick snapping action, termed GSA, or "glochidial snapping action." The number of glochidia per conglutinate was estimated by suspending the glochidia in 1 L of water. While stirring, ten 1 mL subsamples were removed with a volumetric pipette and glochidia from each subsample were counted under magnification on a plankton wheel to estimate total glochidia per subsample. The average number in the subsamples was multiplied by 1000 to estimate the number of glochidia in 1 L or the number of glochidia per conglutinate. Mussels were separated from one another with screen enclosures, allowing ample water flow while isolating the mussels and conglutinates from each other. Viable glochidia were placed in a beaker and held at 19 ^{o}C, and viability was tested every 24 hours with a salt test. A subsample was removed from the beaker and placed in a Petri dish, where the glochidia were exposed to a salt solution. They were considered viable if they snapped shut. This was repeated until glochidia ceased to exhibit any sign of viability.

Results

E. sloatianus were found in a variety of habitats within the Flint River, from hard limestone bottoms at Lake Worth Dam in Dougherty County, to soft muddy substrates at Philema Shoals, Lee County, to coarse stable sand near Montezuma Bluffs, Macon County. At all three locations, there was the presence of moderate stream flow.

During weekly visits to the three sites in the Flint River from July 2009 to February 2010, no gravid mussels were observed. On March 26, 2010, two mussels collected from

the Lake Worth Dam site exhibited early signs of gravidity. *E. sloatianus* observed in the river at all three sites as well as in captivity released conglutinates from April through June. Weekly checks of mussels showed greatly reduced or an absence of gravidity by the beginning of June, and all gravid mussels in captivity had released all their conglutinates by the middle of June. The period of gravidity was determined to be from late-March through mid-June when water temperatures ranged from 15°C to 25°C (Figure 4).

Temperatures were between 14.7-15.0 °C when conglutinates were initially observed in the lab. Gravid *E. sloatianus* in captivity were initially observed releasing 1 to 8 conglutinates that were only 0–50% glochidia. These were slightly smaller than mature conglutinates and did not break apart as readily as a fully developed conglutinate with a higher glochidia to egg ratio. These early conglutinates were termed "pre-release" and were followed by the main release one to two days later. Mature conglutinates, in contrast, showed approximately 95% glochidia and 5% eggs when placed in Petri dish and examined under a dissecting scope. A full release, on average, consisted of 214 conglutinates over an average period of 70 days across the ten mussels used (Figures 2 ,3, & Table 1).

Conglutinates were on average 2.0 cm long and 0.5 cm wide (n=10) and were two to three layers thick. Each layer was equivalent to the thickness of 1-2 glochidia; the layers were attached at one end, which appeared to give the conglutinate a distinct swimming action when floating in the water column. The conglutinates of *E.sloatianus* are bright white when released, apparently mimicking the fry of a prey fish. Once they were released, they settled to the substrate in the absence of stream flow. Each of the 2 to 3

layer thick conglutinates contained approximately 10,000-25,000 glochidia, totaling approximately 2.1–5.4 million glochidia per mussel.

It was not clear why the individual mussels varied in the number of conglutinates they produced. A Pearson product moment correlation showed that there was no relationship between mussel weight and the number of conglutinates they produced ($r=0.261$, $n=10$, $P=0.720$).

Data collected on the viability of glochidia suggests that viability remained constant for a period of 72 hours. After 72 hours, the viability of the glochidia declined rapidly over the next 5 hours to 0%.

Discussion

Elliptoideus sloatianus are considered tachytictic or short-term brooders who spawn in early spring and release glochidia later in the spring continuing through midsummer (Watters 1995). This was evident in this study, as the appearance of gravidity occurred rapidly in the form of swollen gills that changed color from pink to opaque white as the level of gravidity increased. It appeared water temperature was the cue that triggers the release of the fertilized glochidia. In the field, conglutinates were not released until temperatures were at least 14.5-15°C.

All of the mussels observed in captivity initially released a few conglutinates that were underdeveloped, containing mostly eggs, which did not break apart very easily. Conglutinates released after the initial release were larger, contained mostly mature glochidia, and readily broke apart. On occasion, some mussels released the majority of their conglutinates in just a few minutes or, in one case, in a single expulsion. Sometimes

mussels became totally covered with a mass of fragmented conglutinates. The total glochidia per mussel ranged from 2.1–5.4 million glochidia. These values seem high in comparison to other mussel species (Davis and Layzer 2012), however, the mussels used for this study were quite large (Table 1). When released, mature conglutinates remained viable for up to 3 days, suggesting that *E. sloatianus* benefits from water currents as a means for glochidial dispersal coupled with the luring effect of the floating conglutinates. O'Brien (1997) had similar glochidia viability results. This strategy gives the conglutinate a greater chance of contacting a host while distributing the glochidia away from its maternal origin.

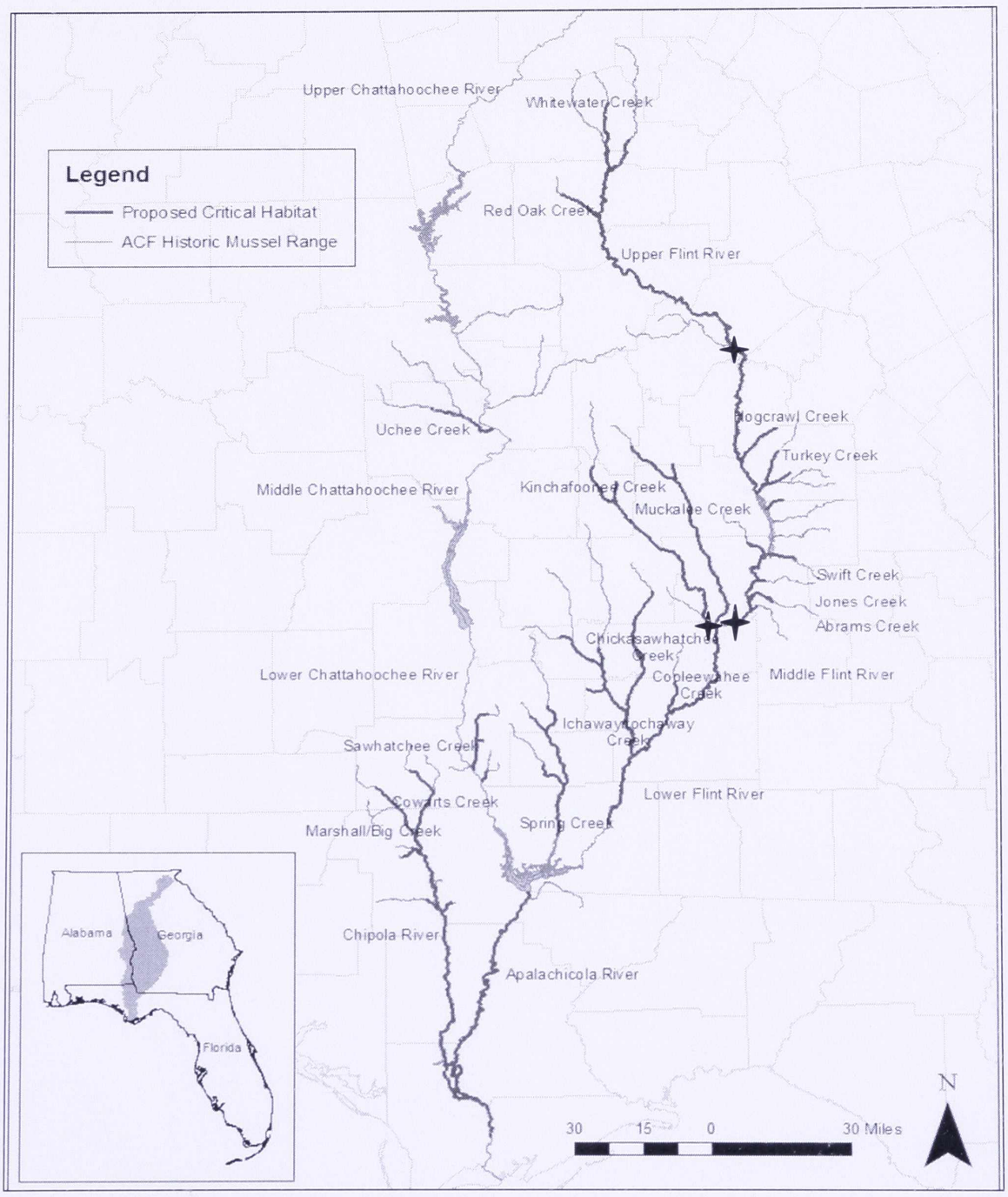

Figure 1 Map of the ACF drainages showing critical habitat and conservation areas represented by the thicker lines, proposed in 2003 by The US Fish and Wildlife Service in response to the 1998 federal listing of 7 mussel species within the ACF basin. The three 4-point stars represent the three gravidity study sites and the mussel collection site. (Map courtesy of U.S. Fish and Wildlife Service 2003 Recovery Plan for the Listed Mussels in the ACF)

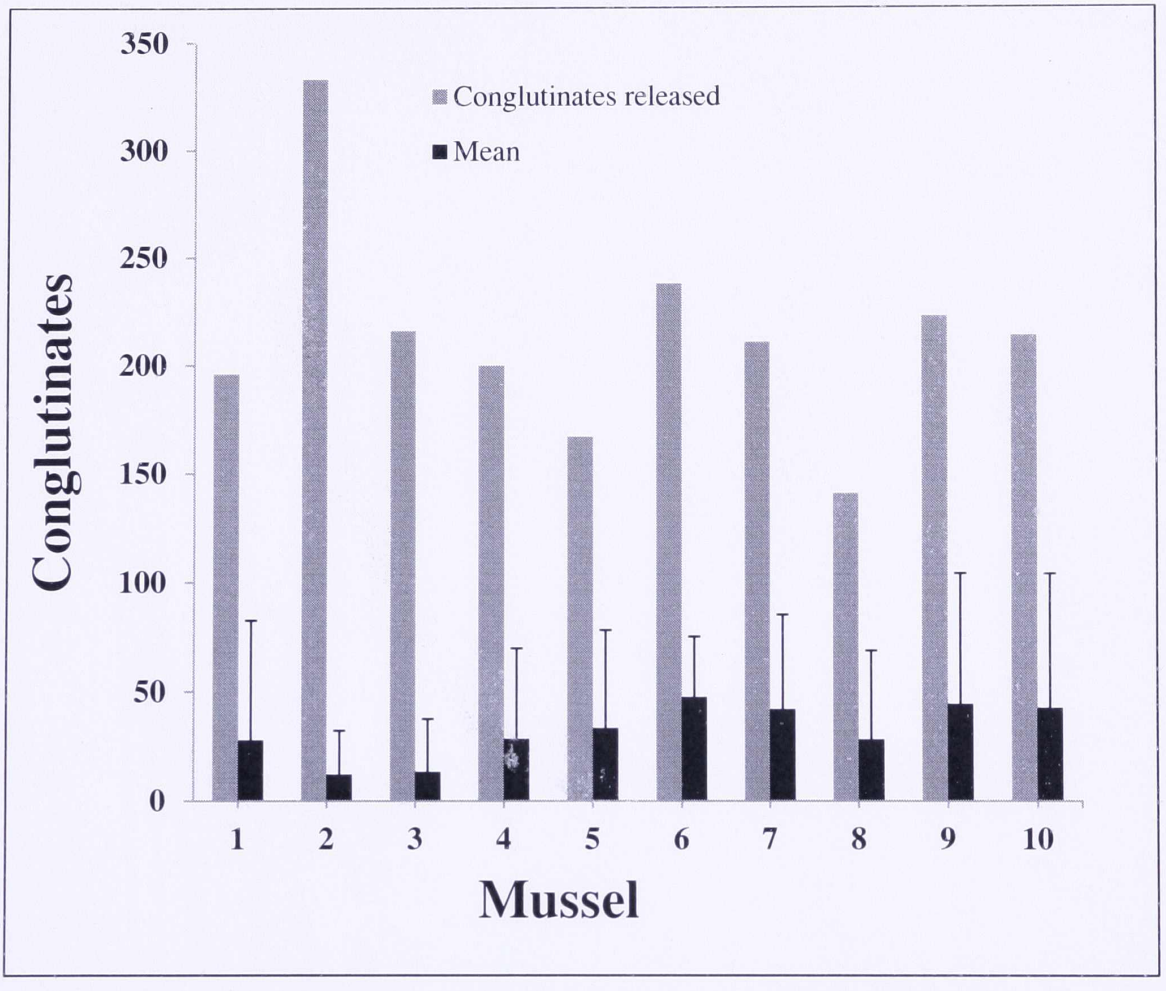

Figure 2 The mean (+/- 1 S.D.) and total conglutinates released (includes pre-release along with the "main" release) for the ten mussels of *Elliptoideus sloatianus*.

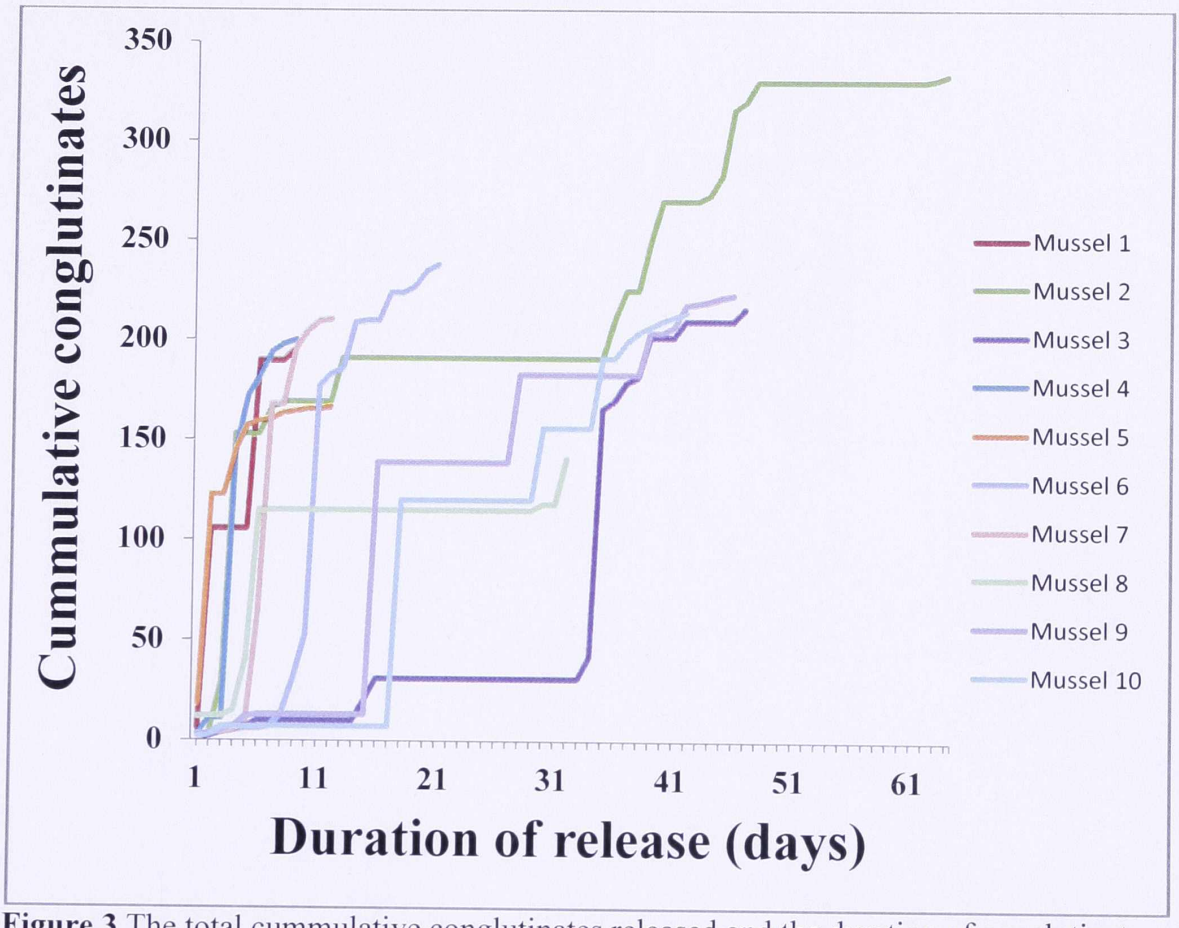

Figure 3 The total cummulative conglutinates released and the duration of conglutinate release in days of 10 mussels used in host trials for *E. sloatianus*.

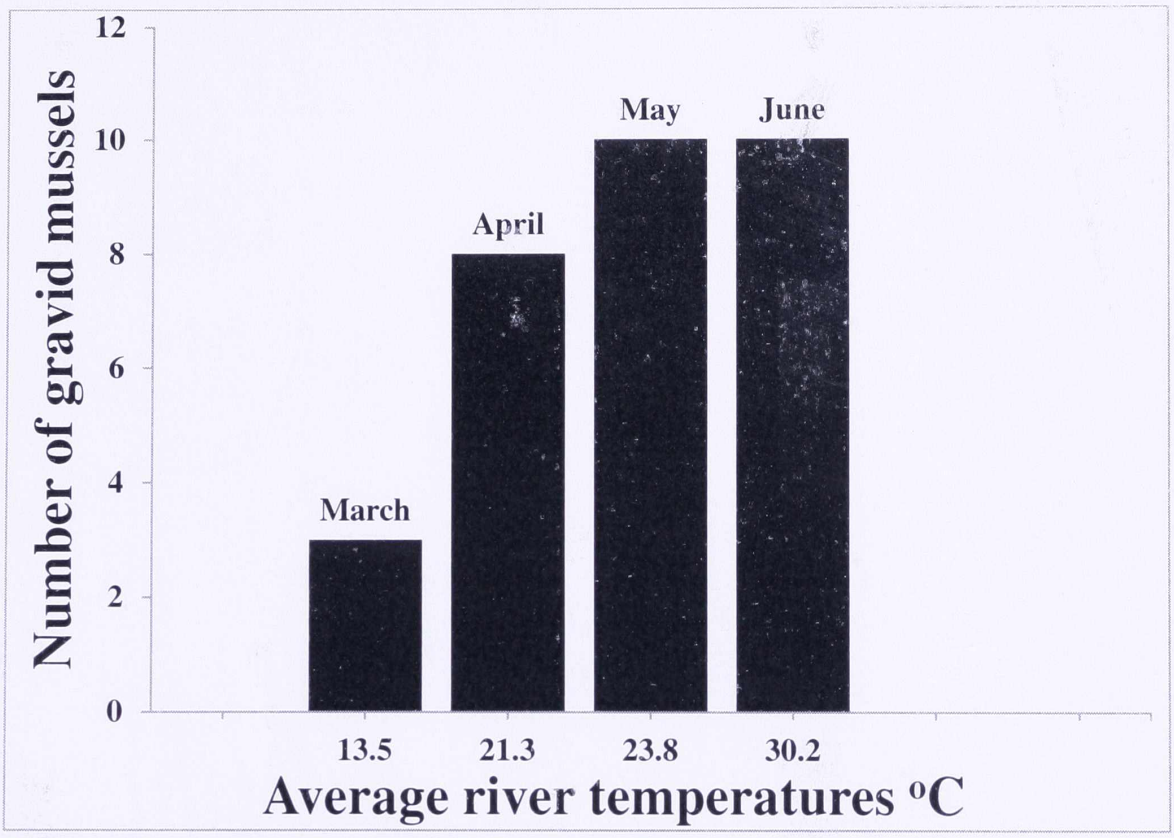

Figure 4 The number of gravid mussels (n=10) across the average monthly river temperates (n=4 months, March, April, May, and June 2010) for the 3 study sites in the Flint River.

Table 1 The weight, length, height, width, the total, mean, (+/-1 S.D.) standard deviation of conglutinates release and the duration of release in days across the ten mussels observed in *Elliptoideus sloatianus* gravidity study.

	Mussel									
Trait	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
weight (g)	793	962	1097	824	866	928	955	942	922	959
length (mm)	180	185	190	190	170	178	193	184	187	188
height (mm)	90	100	110	100	110	115	116	105	110	95
width (mm)	61	64	66	59	64	60	62	63	61	62
conglutinates released	196	333	216	200	167	238	211	141	223	214
mean	28	12.3	13.5	28.6	33.4	47.6	42.2	28.2	44.6	42.8
(+/ - 1 S.D.)	54.6	20	24.2	41.3	44.8	27.6	43.1	40.7	59.6	61.4
duration of release in days	9	63	46	9	2	38	13	37	45	33

Appendix

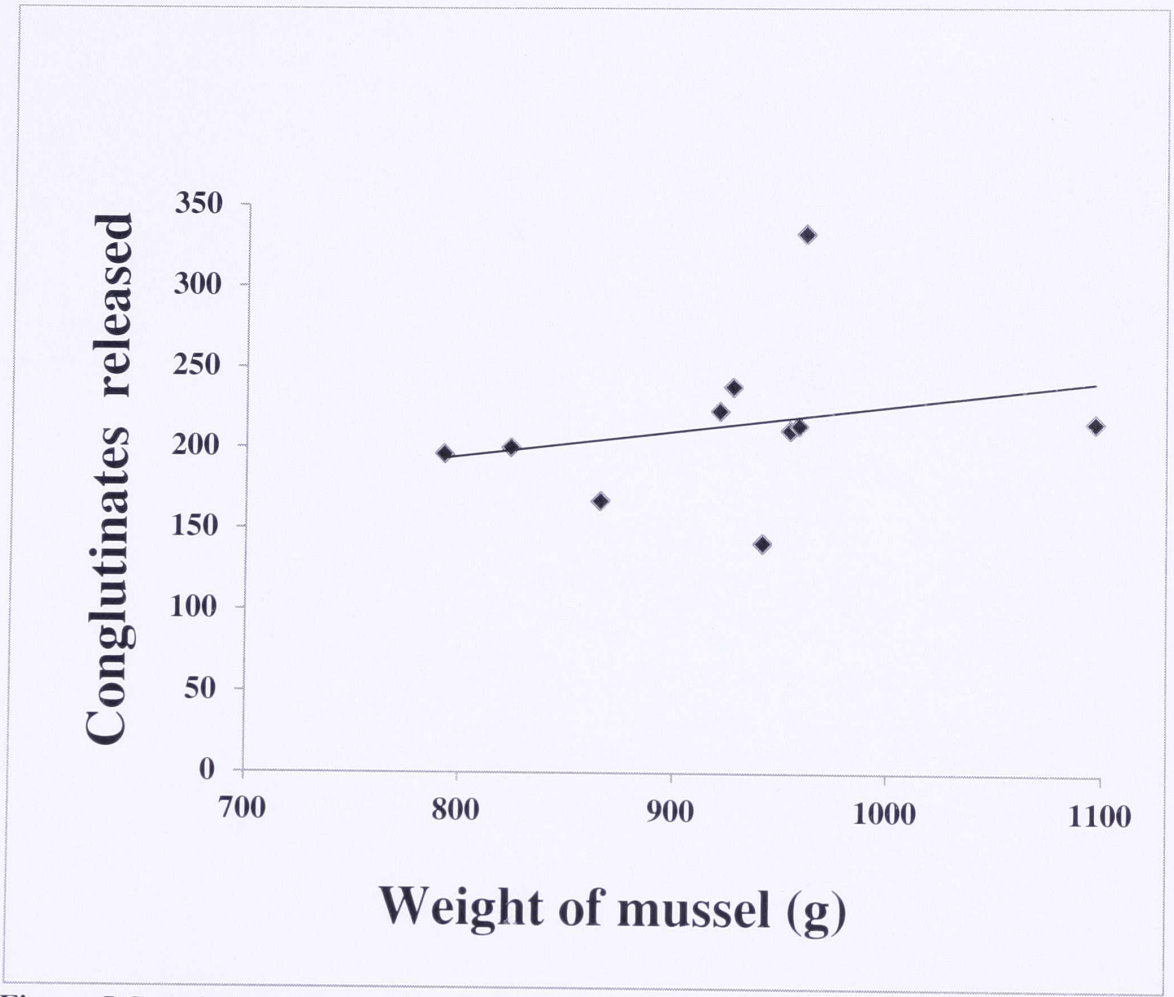

Figure 5 Correlation between the total conglutinates released and the weight of the 10 mussels used in *E. sloatianus* host trials. Pearson product-moment correlation found no significant relationship between mussel weight and number of conglutinates released.

Figure 6 Scanning electron microscope images of *E. sloatianus* glochidia.
Photo: Christine O'Brien.

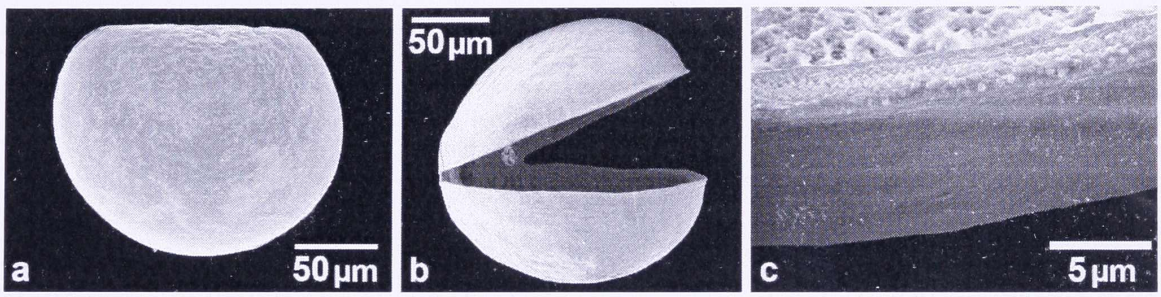

Figure 7 Image showing 2 *E. sloatianus* conglutinates with a U.S. quarter for reference Photo: USFWS

Figure 8 Image showing the 3 layers of *E. sloatianus* conglutinate.
Photo: USFWS

Figure 9 Magnified image showing *E. sloatianus* conglutinate.
Photo: USFWS

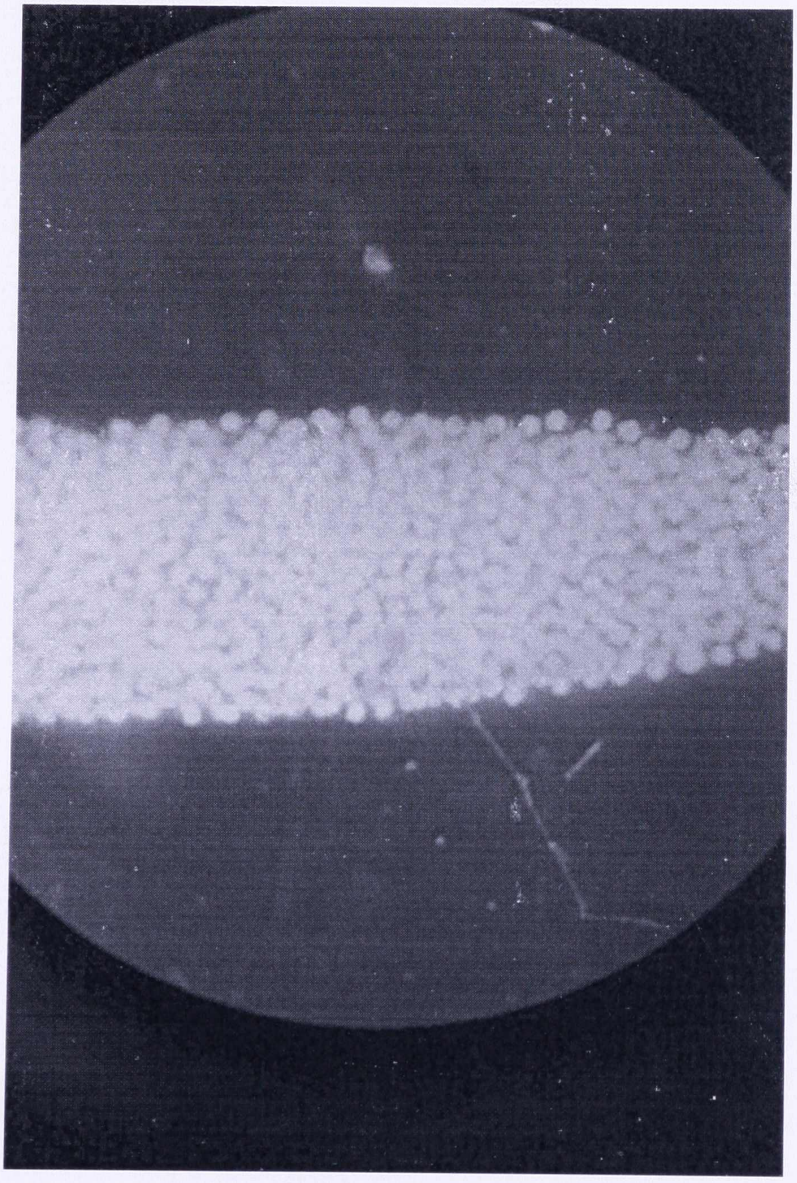

Table 2 Average monthly water quality parameters for three study site locations on the Flint River from July, 2009 through June, 2010.

Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Year	2009						2010					
Lake Worth Dam												
Temp °C	29.2	28.8	21.6	16	10.7	9.8	9	9.2	15.5	22	24	31.5
pH	7	7.5	7	6.5	7	6.5	6.8	7	6.8	6.5	7	6.8
NH^3 (mg/l)	0.5	0.2	0	0	0	0	0	0	0	0.2	0.2	0
Alkalinity(ppm)	104	64	56	60	62	64	68	80	66	54	48	56
Hardness (ppm)	68	62	54	62	58	60	60	74	60	48	44	54
Nitrites (mg/l)	0.05	0	0	0	0	0	0	0	0	0	0.05	0.05
Montezuma Bluff												
Temp °C	26.2	28.7	21	15.7	11	9.5	9.2	9.6	13	20.5	23.5	29.5
pH	7	6.8	6.5	7	6.5	7	6.8	7	7	7	7	7
NH^3 (mg/l)	0.5	0.2	0	0	0	0	0	0	0	0	0.2	0.2
Alkalinity(ppm)	20	18	20	24	26	28	22	24	22	24	20	10
Hardness (ppm)	14	20	20	20	22	24	20	20	20	22	18	15
Nitrites (mg/l)	0.05	0	0	0	0	0	0	0	0	0.05	0.05	0
Philema Shoals												
Temp °C	26	27.7	20.8	15.1	10.2	9.7	9.5	9	12	21.5	23.8	29.5
pH	7.5	7	7	7	7	6.5	6.5	7	6.5	7	6.5	7
NH^3 (mg/l)	0.2	0.5	0	0	0.2	0	0	0	0	0.2	0.2	0.2
Alkalinity(ppm)	48	22	40	46	46	40	44	24	22	20	22	10
Hardness (ppm)	40	16	40	44	38	44	38	28	22	22	20	10
Nitrites (mg/l)	0	0.05	0	0.05	0	0	0	0	0	0	0.05	0

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